

Figure 1

1 MQTCPLAEPG HVSQALGTLL FLAASLSAQN EGWDSPICTE GVVSVSWGEM
51 TVMSCNISNA FSHVNIKLRH HGQESAIFNE VAPGYFSRDG WQLQVQGGVA
101 QLVIKGARDS HAGLYMWHLV GHQRNNRQVT LEVSGAEPQS APDTGFWPVP
151 AVVTAVFILL VALYMFAYWR CRCSQORREK KFFLLEPQMK VAALRAGAQQ
201 GLSRASAELW TPDSEPTPRP LALVFKPSPL GALELLSPQP LFPYAADP*

Figure 2

12 promoter (1-195) and cDNA (196-2180) sequence

1 ATTCCTGCTT CCTTTAGCGT GAACGCGGGT GCGGTGCCTC CCGTGAAATA
51 ATAAATTAC CGTCACGCTT GTTGTGAACG CGGGTGGTTC CCGAAACTTG
101 GAGGCTTCCC GTAAACCCAG CTCCTTCCTC ATCTGGGAGG TGGGTCCCGC
151 GCGGGTCCGC CGCCTCCTCC CTGGCCCCCTC CCTCTCGTGT CTTTCATTTT
201 CCTGGGGCTC CGGGGCGCGG AGAAGCTGCA TCCCAGAGGA GCGCGTCCAG
251 GAGCGGACCC GGGAGTGTTT CAAGAGCCAG TGACAAGGAC CAGGGGCCCA
301 AGTCCCACCA GCCATGCAGA CCTGCCCCCT GGCATTCCCT GGCCACGTTT
351 CCCAGGCCCT TGGGACCCTC CTGTTTTTGG CTGCCTCCTT GAGTGCTCAG
401 AATGAAGGCT GGGACAGCCC CATCTGCACA GAGGGGGTAG TCTCTGTGTC
451 TTGGGGCGAG AACACCGTCA TGTCTGCAA CATCTCCAAC GCCTTCTCCC
501 ATGTCAACAT CAAGCTGCGT GCCACGGGC AGGAGAGCGC CATCTTCAAT
551 GAGGTGGCTC CAGGCTACTT CTCCCGGGAC GGCTGGCAGC TCCAGGTTCA
601 GGGAGGCGTG GCACAGCTGG TGATCAAAGG CGCCCGGGAC TCCCATGCTG
651 GGCTGTACAT GTGGCACCTC GTGGGACACC AGAGAAATAA CAGACAAGTC
701 ACGCTGGAGG TTTCAGGTGC AGAACCCAG TCCGCCCCCTG ACACTGGGTT
751 CTGGCCTGTG CCAGCGGTGG TCACTGCTGT CTTTCATCCTC TTGGTCCGCTC
801 TGGTCATGTT CGCCTGGTAC AGGTGCCGCT GTTCCCAGCA ACGCCGGGAG
851 AAGAAGTTCT TCCTCCTAGA ACCCCAGATG AAGGTCGCAG CCCTCAGAGC
901 GGGAGCCCAG CAGGGCCTGA GCAGAGCCTC CGCTGAACTG TGGACCCCAG
951 ACTCCGAGCC CACCCCAAGG CCGCTGGCAC TGGTGTTCAA ACCCTCACCA
1001 CTTGGAGCCC TGGAGCTGCT GTCCCCCCCCA ACCCTTGTTT CCATATGCCG
1051 CAGACCCATA GCCGCCTGCA AGGCAGAGAG GACACAGGAG AGCCAGCCCT
1101 GAGTGCCGAC CTTGGGTGGC GGGGCCTGGG TCTCTCGTCC CACCCGGAGG
1151 GCACAGACAC CGGCTTGCTT GGCAGGCTGG GCCTCTGTGT CACCCACTCC

12 promoter (1-195) and cDNA (196-2180) sequence

1201 TGGGTGCGTG CAGACCCTTC CCCTCCACCC CCCAGGTCTT CCAAGCTCTG
1251 CTTCCTCAGT TTCCAAAATG GAACCACCTC ACCTCCGCAG CACCCGACTT
1301 ACCAGGACGC ATGCCCCCTCC CTCTGCCCTC ATCAAACCCA CAGACCCGGA
1351 CTCCCTTTCT GCCACCCAG GCTGGTCCGG CCCAGGTGT GGGGTCCGCT
1401 CTCTCCACTC CCAGGGCTCC GCGCCCAAGT GAGGGGGCCC CTGCCGGAGC
1451 CTCAGACACA CTGGAGTTCA GGGCTGGGGG GGCCTTGGCA CATACCTGTC
1501 CCTTGGCTAT GAGCAGGCTT TGGGGGCCCT TCCGCGGCAG CCCCAGGGGC
1551 CGAGGTA $\bar{G}\bar{G}$ TCTGGGGGCT TAGAGGCTGG GATGGCTCCT GGCCCCACCG
1601 CCAGGGGGCA AGCGCAGGCC GGGCTGGGAG GCGGCGGCGG CGGCTCGGGC
1651 TGGGGGGTCA GGTGGACGCT GCCTCCGGGG CTGGTCGCGC ATCCCTCAGT
1701 CCCTCGGCCA CCCGGGGGTC GCTCCCTCGT GCCCACC GCA CCTGCCGAGC
1751 CTCTTTGGAC CCAGATCTGT TCATGCTTTT GTCTTCGTCA CTGCGGCGGG
1801 GCCCTTTGAT GTCTTCATCT GTATGGGGTG GAAAAATCAC CGGGAATCCC
1851 CCTTCAGTTC TTTGAAAAAG TTCCATGACT CGAATATCTG AAATGAAGAA
1901 AACAAACCGA CTCACAAACC TCCAAGTAGC TCCAAATGCA ATTTTAAAA
1951 TGAAAAACAA AAATCTGAAA GAAACGTCTT TAGTGGCTTT AAGCCCCAAA
2001 ACGTCCCTAA GCGTCCTCG AGATGAAGAC GGGGGGGAGC CCCAGCCAGG
2051 TGGAGACCCC GCAGGACGCG GCGGCGCCCG GTGACCGAGG CCTCGCACAG
2101 CCGGCCGCCC TGAGGGTCGG GCCGAGCCAG GGTCCAAGAG GGGCGCGTTT
2151 GTGTCTCGGG TTAAAATAAG GTTCCGTCCG

Figure 3 : K12 Expression

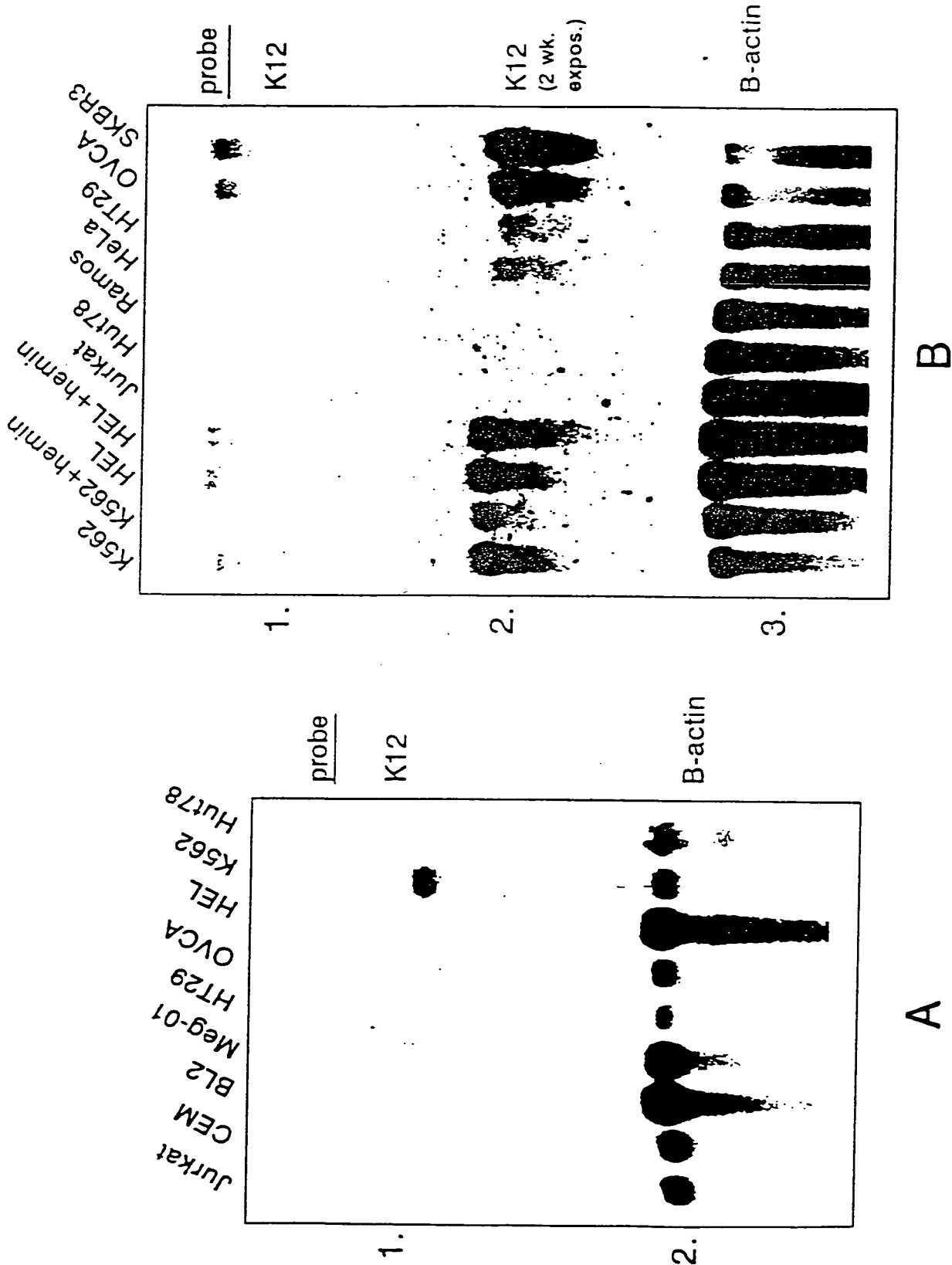
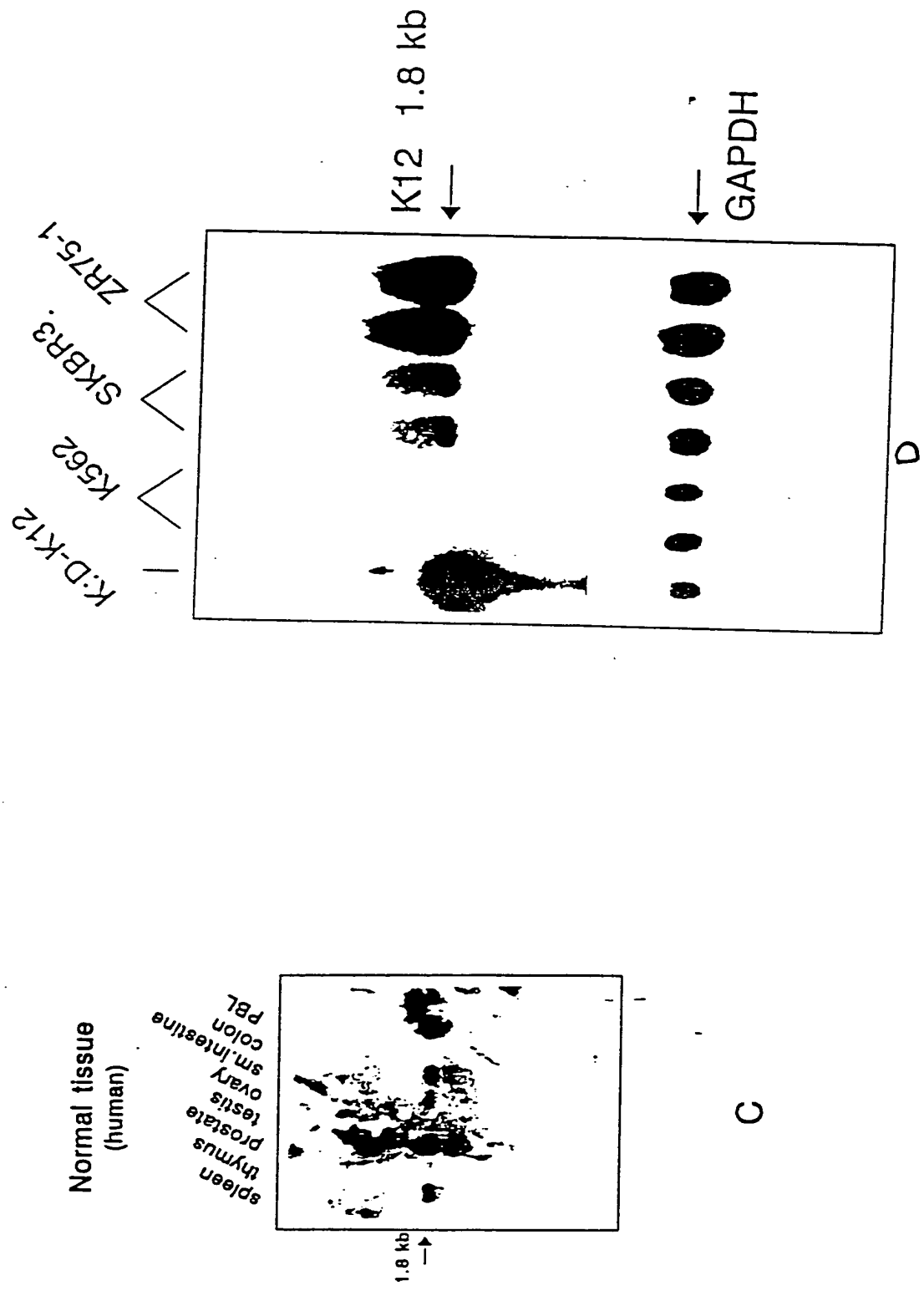


Figure 3 (cont)

Total RNA Northern



Western Blot Probed with Antiserum to K12

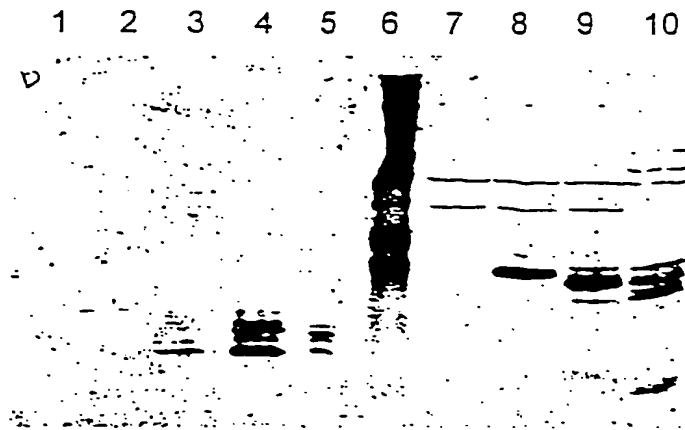


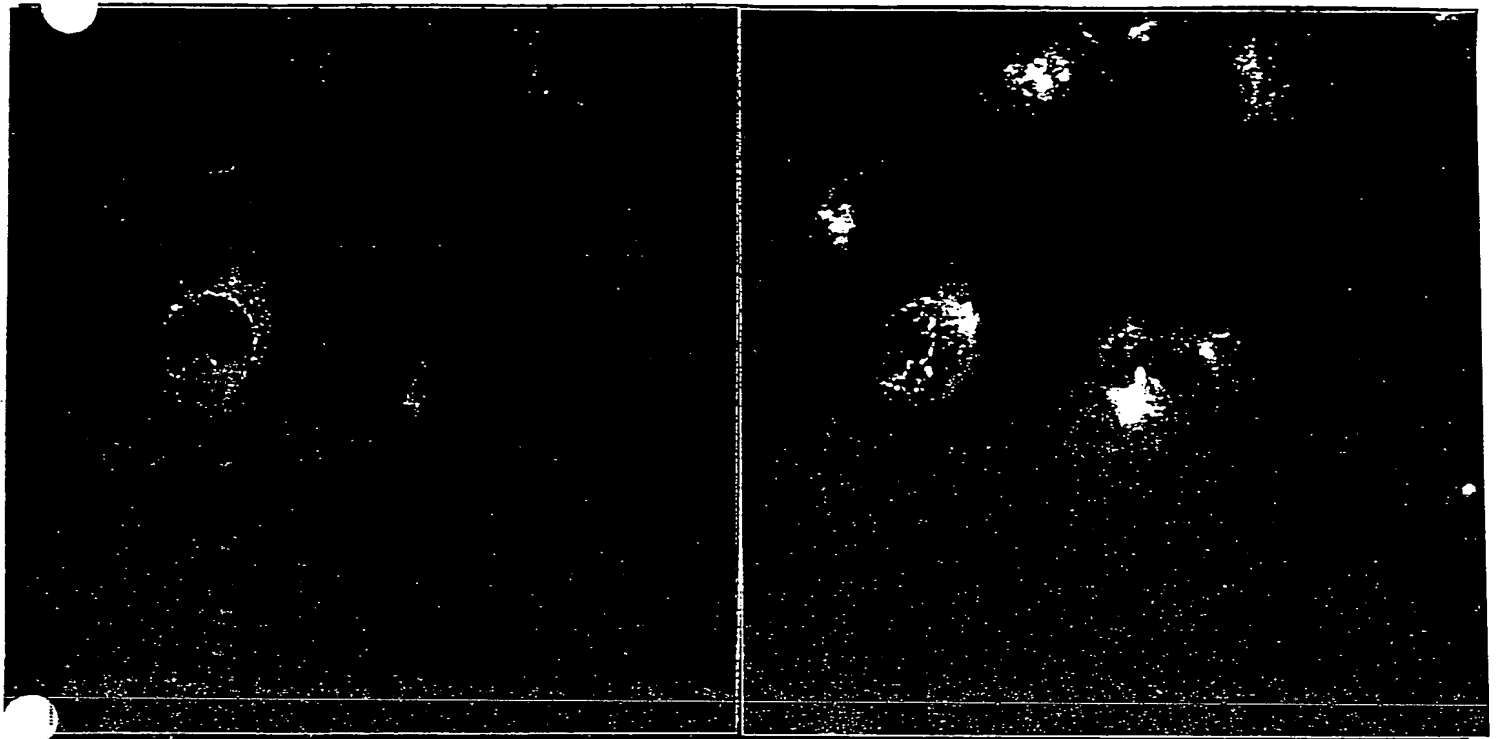
Figure 4. Western blot probed with antiserum to K12. Concentrated media from K562 cells transfected with:

- 1) empty vector
- 2) K12 and 7 amino acid flag
- 3) K12 with C terminus addition
- 4) Full length K12
- 5) ZR75-1 cells (not transfected)
- 6) Molecular weight standards (smallest is 32 kDa)

Soluble protein extracts from K562 cells transfected with :

- 7) empty vector
- 8) K12 with 7 amino acid flag
- 9) Full length K12
- 10) ZR75-1 cells (not transfected)

Figure 5: Subcellular Localization of K12 to the Golgi



A

B

Figure 5

Same field of view of ZR75-1 cells that were grown on slides, acetone-fixed and double stained with,

A: antigen -purified anti-K12 polyclonal antibody followed by FITC-conjugated goat anti-rabbit IgG secondary antibody.

B: Rhodamine conjugated Wheat Germ Agglutinin (an immunochemical marker for Golgi bodies)

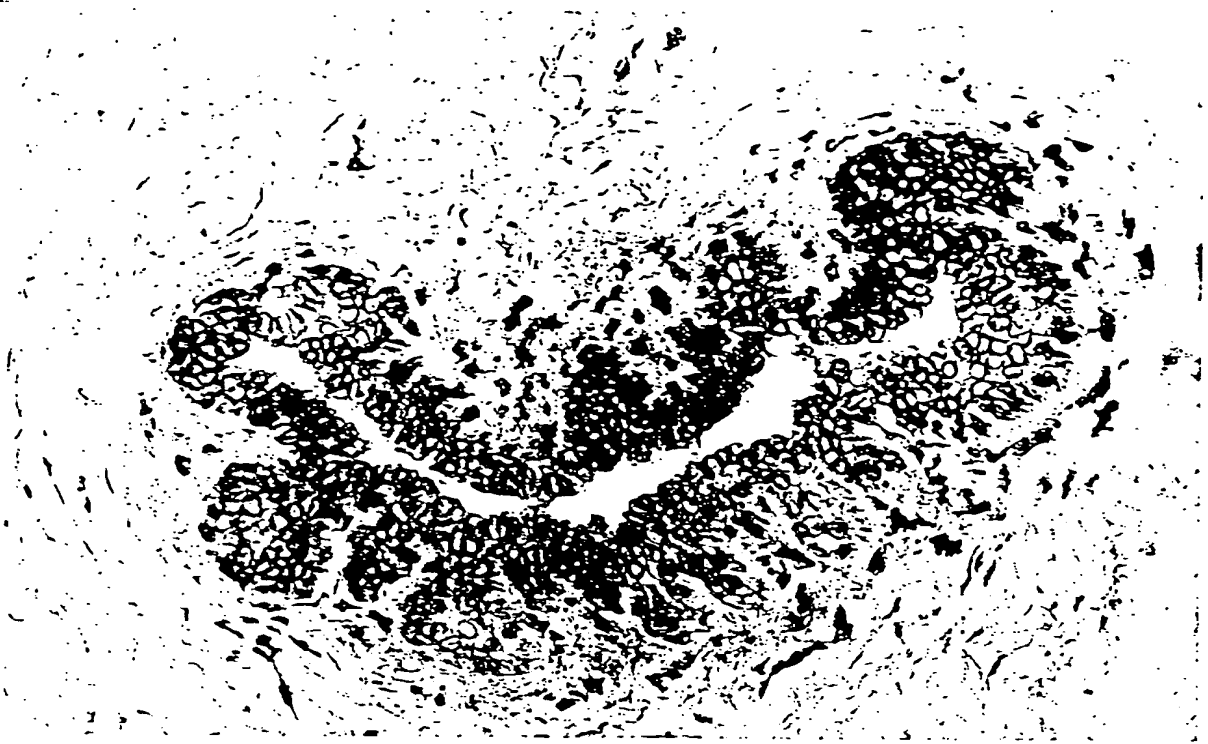


Figure 6 Immunoperoxidase staining of normal breast tissue, A, and colloid breast carcinoma, B, with monoclonal antibody 7C3 against K12. Panel C is a isotype matched P3 control. Dark brown staining reflects monoclonal antibody binding to K12 antigen.

Conditioned Media Proliferation Assay

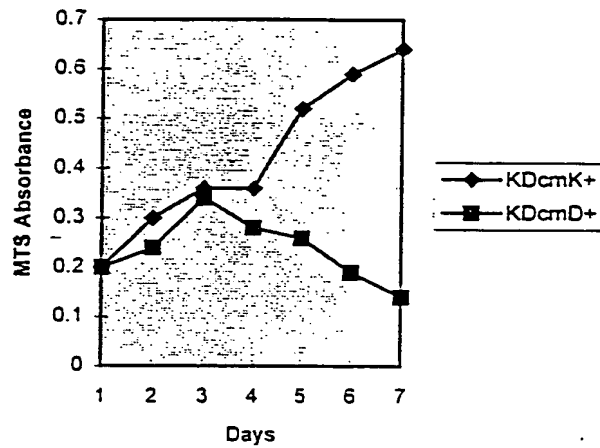


Figure 7: Growth Curves for K562 cells grown in conditioned media from :

KDcmK+, K562 cells secreting K12 into the media, or

KDcmD+, K562 cells transfected with an empty vector and producing no detectable K12 in media.